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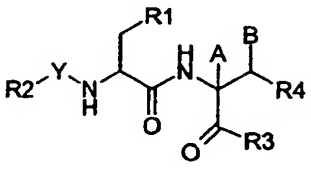
(54) Title: CYSTEINE PROTEASE INHIBITORS



(I)

(57) Abstract: This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins. A compound of formula (I) is described or a pharmaceutically acceptable salt, hydrate or solvate thereof. Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

Cysteine Protease Inhibitors

This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins.

Background to the Invention

The cathepsin family (C1) of lysosomal cysteine (or thiol) proteases is a subset of the papain superfamily (clan CA of cysteine proteases) and includes cathepsin B, H, K, S, L and the recently discovered cathepsins O, O2/K, V, X, Z and W (lymphopain). Related enzymes also regarded as cysteine proteases are the cytoplasmic Ca^{2+} dependent calpains (family C2). Cysteine proteases are classified both functionally and according to the nature of their active site, which has a thiol residue. They differ in substrate specificities and other enzymatic activities, these differences probably arising from evolutionary divergence.

The known cathepsins are synthesized on membrane bound ribosomes, transferred to the endoplasmic reticulum, then to the Golgi apparatus and finally to the lysosome and endosomes. They have an important function in regulation of intracellular protein metabolism, mobilisation of tissue proteins and conversion of proenzymes, prohormones and neuropeptides into biologically active molecules. The cathepsins are believed to be involved in a number of diseases.

Cathepsin K can be secreted into the extracellular space and is involved in bone and cartilage remodelling. Cathepsin K is implicated in the pathogenesis of osteoporosis. Cathepsin K inhibitors can prevent osteoporosis in animal models (PNAS.1997. 94:14249-14254). Cathepsin L inhibitors have also been shown to inhibit osteoporosis (Bone, 1997. 20:465-471).

Cathepsin B and other cysteinyl cathepsins have also been shown to be released extracellularly by various tumour cells and are thought to play a role in tumour invasion (Journal of cellular Physiology. 1992. 150:534-544).

The cysteinyl cathepsins have also been shown to play a role in rheumatoid arthritis (Arthritis and Rheumatism 1994. 37:236-247) and neuronal and cardiac ischaemia (European Journal of Neuroscience. 1998. 10.1723-1733).

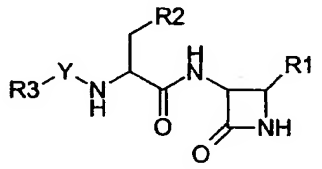
Cathepsins S and L both play a role in the generation of free MHC class II molecules capable of binding antigenic peptides in the endosomes. These class II/peptide complexes move to the cell membrane and are involved in T lymphocyte activation. Inhibitors of Cathepsin S have been shown to inhibit allergic immune responses (Journal of Clinical Investigation. 1998. 101:2351-2363).

In addition to their role in the above diseases, cysteinyl cathepsins play a major role in the pathogenesis of infectious diseases. For example, cysteinyl cathepsins are used by the protozoal parasites Plasmodium (malaria) and Trypanosoma (Chagas Disease) to invade the human host and

cysteinyl cathepsin inhibitors can inhibit experimental disease in both cases (Antimicrobial agents and chemotherapy. 1998. 42:2254-2258; Journal of Experimental Medicine. 1998. 188:725-734). Cysteinyl cathepsins are also virulence factors for several pathogenic bacteria.

A recent review (Annu. Rev. Physiol. 1997. 59:63-88) describes the state of the art of cysteine proteases, including the cathepsins, and their presumed biological functions. Other reviews deal with cathepsin B inhibitors as potential anti-metastatic agents (Exp. Opin. Ther. Patents,1998, 8: 645-672), and cathepsin K inhibitors as potential treatments for osteoporosis (Exp. Opin. Ther. Patents, 1999, 9: 683-644).

International patent applications WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 describe, inter alia, classes of cysteine protease inhibitors which may be represented by formula (IA):



(IA)

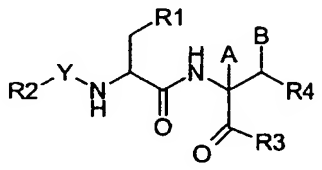
wherein Y, R_1, R_2 and R_3 represent the groups found in corresponding positions of the compounds disclosed in those publications. These known compounds are azetidin-2-ones which are monosubstituted at positions 3 and 4.

Brief Description of the Invention

The present invention makes available a new class of cysteine protease inhibitors which differ in structure from those disclosed in WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moeity, as more fully explained below. These compounds are useful for the treatment of diseases mediated by cysteine protease activity, for example muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, and protozoal or bacterial disease.

Detailed Description of the Invention

According to the present invention, there is provided a compound of formula (I)



(I)

wherein:

Y represents -C(O)- or -S(O_2)-;

R_1 represents a radical of formula R_6-(ALK)$_p$-(Z)$_n$-(ALK)$_q$- wherein Z represents -O- or -S-, ALK represents a divalent C_1-C_3alkyl or halogen-substituted C_1-C_3alkyl

radical, p and q are independently 0 or 1, n is 0 or 1 when q is 1 and n is 0 when q is 0, and R_6 is hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group; or R_1 together with the carbon atom to which it is attached forms a cycloalkyl ring;

R_2 represents $-OR_5$ or $-R_5$;

R_5 represents a radical of formula R_7-$(A)_t$- wherein t is 0 or 1; A represents (i) an optionally substituted divalent C_1-C_6alkyl, radical which may be interrupted by one or more non-adjacent -O-, -S- or -NH- linkages, or (ii) a divalent C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R_7 represents hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

R_3 represents (i) an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR_8 or $N(R_8)_2$ or (iii) OR_8 wherein R_8 represents hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl or aryl;

A and B taken together represent a bond and R_4 represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R_4 each independently represents a hydroxy or substituted hydroxy group;

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

As used herein the term (C_1-C_6)alkyl or lower alkyl means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, and hexyl. Similar terms such as "(C_1-C_3)alkyl" are to be interpreted similarly.

As used herein the term C_2-C_6alkenyl" means a straight or branched chain alkenyl moiety having from 2 to 6 carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl. Similar terms such as "(C_2-C_3)alkenyl" are to be interpreted similarly.

As used herein the term "C_2-C_6 alkynyl" means a straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-

hexynyl and 5-hexynyl. Similar terms such as "(C_2-C_3)alkynyl" are to be interpreted similarly.

As used herein the term cycloalkyl means a saturated alicyclic moiety having from 3-7 carbon atoms and includes, for example, cyclohexyl, cycloheptyl, cyclopentyl, cyclobutyl and cyclopropyl.

As used herein the term "halogen" means fluoro, chloro, bromo or iodo.

As used herein the term "aryl" refers to a mono-, bi- or tri-cyclic, substituted or unsubstituted, carbocyclic aromatic group, and to groups consisting of two covalently linked substituted or unsubstituted monocyclic carbocyclic aromatic groups. Illustrative of such groups are phenyl, biphenyl and napthyl. Examples include C_6-C_{12} aryl groups such as phenyl, biphenyl, naphthyl, tetrahydronaphthyl, dihydronaphthyl, and cyclohexyl phenyl.

As used herein the unqualified term heterocyclyl or heterocyclic means a 5-7 membered heterocyclic ring, which may be aromatic or non-aromatic, containing one or more heteroatoms selected from S, N and O, and optionally fused to a benzene or hetero-atom containing ring. The term therefore includes C_1-C_{11} heterocyclic groups containing 1-4 heteroatoms selected from nitrogen, sulfur or oxygen. Examples include thienyl, pyridyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3,4-tetrazolyl, imidazolyl, quinolinyl, isoquinolinyl, indolyl, pyrimidinyl, benzofuranyl, benzothienyl, morpholinyl,

thiomorpholinyl, piperazinyl, piperidinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, pyridylphenyl, pyrimidylphenyl, pyrrolyl, furyl, thienyl, piperidinyl, imidazolyl, oxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, benzimidazolyl, maleimido, succinimido, and phthalimido groups.

As used herein, the term "primary or secondary amino group" means an amino group carrying one or two substituents respectively, for example selected from amino protecting groups, (C_1-C_6)alkyl-X-, (C_2-C_6)alkenyl-X-, (C_2-C_6)alkenyl-X-, aryl(C_1-C_6)alkyl-X-, aryl(C_2-C_6)alkenyl-X-, aryl(C_2-C_6)alkenyl-X-, heterocyclic(C_1-C_6)alkyl-X-, heterocyclic(C_2-C_6)alkenyl-X-, heterocyclic(C_2-C_6)alkenyl-X-, wherein -X- represents a bond or a carbonyl -C(O)-, sulphonyl -S(O_2)-, or oxycarbonyl -O-C(O)- group, and wherein any of the foregoing may be substituted. The term "secondary amino group" also means a substituted or unsubstituted cyclic amino group, such as piperidyl ,morpholinyl, thiomorpholinyl, piperazinyl, pyrrolidynyl or azetidinyl.

As used herein, the term "substituted hydroxy group" means a protected hydroxy group or a hydroxy group substituted by, for example, any of the substituents listed in the preceding paragraph as substituents of primary or secondary amino groups except those wherein X is an oxycarbonyl -O-C(O)- group.

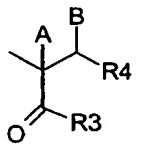
As used herein in contexts other than "substituted hydroxy group", the unqualified term "substituted" as applied to a group or radical means substituted with 1, 2, or 3 substituents selected from

(C_1-C_3)alkyl;
phenyl;
hydroxy or mercapto;
(C_1-C_3)alkoxy or (C_1-C_3)alkylthio;
phenoxy or phenylthio;
halogen;
trifluoromethyl;
nitro;
cyano (-CN);
carboxyl, and amidated, esterified or protected carboxyl;
amino, mono- or di-(C_1-C_3)alkylamino, or protected amino;
(C_1-C_3)alkylcarbonyl- or (C_1-C_3)alkylcarbonylamino-;
$-CONHR^A$, $-NHR^A$, $-NR^AR^B$, or $-CONR^AR^B$ wherein R^A and R^B are independently (C_1-C_3)alkyl; and
$-NH-C(=NR_9)R_{10}$ wherein R_{10} is amino, mono- or di-(C_1-C_6)alkylamino, protected amino, or (C_1-C_3)alkyl, and R_9 is hydrogen, (C_1-C_3)alkyl, or an N-protecting group.

As used herein the term "protecting group" when used in relation to an amino, hydroxy or carboxylic acid moeity in the compounds of this invention means a group which is used to render the amino, hydroxy or carboxylic acid moeity substantially non reactive, ie to neutralise its

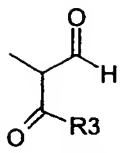
amino, hydroxy or carboxylic acid functionality. In this context, protected amino groups include amido and acylamino, protected hydroxy groups include ethers, protected carboxyl groups include esters, and imidazolyl, indolyl or guanidyl groups may be protected as t-butoxycarbonyl derivatives. These are only examples of the many protecting derivatives known in the art, and others will be known to the skilled man. Such protecting groups are of course well known, eg from the art of peptide synthesis, and are discussed in the widely used handbook by T.W. Greene and P.G.M. Wuts, Protective groups in Organic Synthesis, 2nd Edition, Wiley, New York 1991, and elsewhere in the chemical literature.

As mentioned above, the compounds of the invention differ in structure from those of WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moeity. That substituted carbonylmethyl moeity is the radical (II):



(II)

which may be regarded as notionally derived from the aldehyde radical (III):



(III)

The substituents R_1 and R_2 in the compounds of the invention may be any of the groups falling within the above definitions of R_1 and R_2 which are present in corresponding positions of cysteine protease inhibitors disclosed in WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0. Without prejudice to the generality of the foregoing, in the compounds of the invention:

Y may be, for example, -C(O)-;

R_1 may be, for example, a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy, methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy - phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl; tetrahydroquinolinyl; tetrahydronaphthyl; aminonaphthyl; or acetamidonaphthyl. Presently preferred are phenyl, isopropyl, cyclohexyl and 3-pyridinyl.

R_2 may be, for example, benzyloxy, 3-phenylpropyloxy, 3-phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl, methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-

(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-prop-1-enyl.

R_3 may be, for example, methyl, ethyl, isopropyl, t-butyl, cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl, pyridyl, $-NH_2$, methylamino, dimethylamino, benzylamino, piperidino, morpholino, piperazino, N-methylpiperazino or methoxy, ethoxy, t-butyloxy or phenoxy.

When A and B taken together represent a bond, R_4 may be, for example, $-NH_2$, acetylamino, methylamino, dimethylamino, benzylamino, morpholino, piperidino, morpholino, piperazino or N-methylpiperazino, methoxycarbonylmethylamino, (methoxycarbonyl)-phenethylamino, -OH, methoxy, allyloxy, benzyloxy.

Specific compounds of the invention include those named and characterised in the Examples herein.

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-acrylamide
2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-amino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-acrylamide
2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(morpholin-4-yl)-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(2-hydroxyethylamino)-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-phenylamino-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-piperidino-acrylamide

2E)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide

(2Z)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3,3-dihydroxy-propionamide

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3,3-dihydroxy-propionamide

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3,3-dihydroxy-propionamide

2-[2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido]-3,3-dihydroxy-propionamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-hydroxy-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-N-benzyl-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(4-methylpiperazino)-acrylamide

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(3-tert-butoxycarbonylamino-pyrrolidino)-acrylamide

(2E)-(2S-2-benzyloxycarbonylamino-2-cyclohexymethyl-acetamido)-3-acetamido-acrylamide

(2Z)-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-acetamido-acrylamide

tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate

Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate

2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 2-(morpholino-1-methylenyl)-acetophenone.

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 2-(morpholino-1-methylenyl)-4'-fluoroacetophenone.

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide

(2Z)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide

As stated, the compounds of the invention are inhibitors of cysteine proteases, for example cathepsins B, L, S and/or K. The invention therefore also provides a pharmaceutical composition containing a compound of formula (I) as defined above, and a pharmaceutically acceptable carrier. Also provided is the use of such a compound in the preparation of a composition for inhibiting cysteine protease activity in the body of a mammal suffering a disease mediated by such activity, and a method of treatment of an animal suffering from a disease mediated by cysteine protease activity, which method comprises administering to the mammal a sufficient amount of a compound of formula (I) as defined above to inhibit such activity.

Diseases mediated by cysteine protease activity include muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, and protozoal or bacterial disease.

Compositions with which the invention is concerned may be prepared for administration by any route consistent with the pharmacokinetic properties of the active ingredient(s).

Orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or

gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

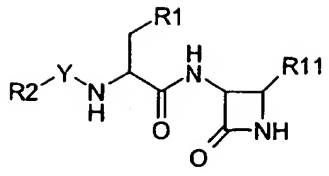
For topical application to the skin, the active ingredient(s) may be made up into a cream, lotion or

ointment. Cream or ointment formulations, which may be used for the drug, are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

The active ingredient(s) may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. Intravenous infusion is another route of administration for the compounds.

Safe and effective dosages for different classes of patient and for different disease states will be determined by clinical trial as is required in the art. It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Compounds of the invention wherein A and B taken together represent a bond and R_4 represents NH_2 may be prepared by treatment of azetidin-2-ones of formula (IV) with ammonium hydroxide.



(IV)

wherein R_{11} is a leaving group such as phenoxy, acetoxy .

Compounds of the invention wherein A and B taken together represent a bond and R_4 represents a primary or secondary amino group may be prepared by treatment of compounds (IV) with a primary or secondary amine, or by appropriate derivatisation of the amino group of the corresponding compounds wherein R_4 is amino.

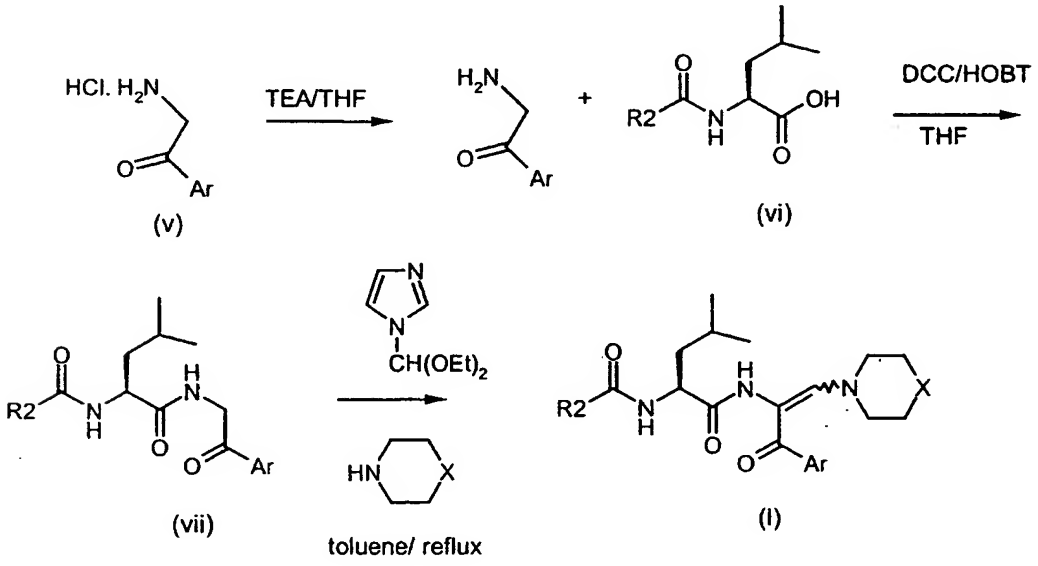
Compounds of the invention wherein A and B taken together represent a bond and R_4 represents a hydroxy group may be prepared by treatment of compounds (IV) with acetic acid, for example at ambient temperatures. Compounds of the invention wherein A represents hydrogen and B and R_4 represents a hydroxy group may also be prepared by treatment of compounds (IV) with acetic acid, but under less forcing conditions than for the alpha-beta unsaturated compounds, for example at low temperatures such as about $0^{o}C$.

Compounds of the invention wherein A and B taken together represent a bond and R_4 represents a substituted hydroxy group or a primary or secondary amino group may be prepared from the corresponding compounds wherein R_4 is

hydroxy or amino by appropriate derivatisation of that hydroxy or amino group. Likewise, compounds of the invention wherein A represents hydrogen and B and R_4 are independently a substituted hydroxy group may be prepared from the corresponding compounds wherein B and R_4 are hydroxy by appropriate derivatisation of one or both of those hydroxy groups.

Compounds of the invention wherein A and B taken together represent a bond and R_4 represents an alkyl, alkenyl, alkynyl, cycloalkyl or aryl may be prepared by the following the synthetic scheme as depicted below in scheme 1.

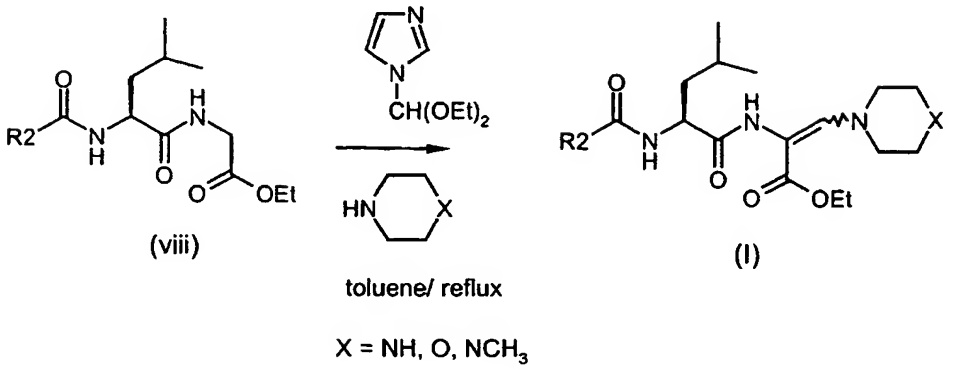
Scheme-1



Compounds of the invention wherein A and B taken together represent a bond and R_4 represents an alkoxy, aryloxy or

cycloalkoxy may be prepared by the following the synthetic scheme as depicted below in scheme 2.

Scheme-2



In the above processes, the reactants are reacted together with solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Depending on the reactants, a solvent will generally be selected from the group consisting of benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, water, pyridine, acetone and the like. Solvent mixtures may also be utilized.

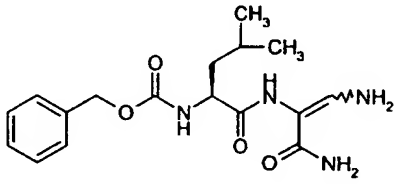
Reaction temperatures generally range from between -70 °C to 150 °C. The preferred molar ratios of reactants are 1:1 to 5. The reaction time range from 0.5 to 72 hours, depending on the reactants.

The azetidine-2-one strating materials (V) may be prepared by literature methods, including those in International patent applications WO 96/32408, WO 98/12176, WO 98/12210.

The following Examples illustrate embodiments of the invention.

Example 1

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amino-acrylamide



A solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (3.0 g, 7.6 mmole) in acetonitrile (50 ml) and 15 ml of ammonium hydroxide (28% NH_3 in water) was stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 1.86 g of the title compound was obtained as white solid.

Yield: 70%

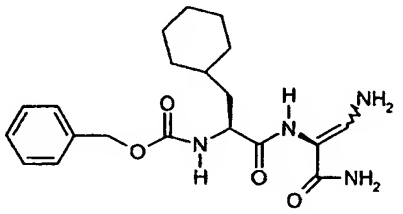
m.p.: 80-90 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.7-1.0 (6H, m), 1.4-1.75 (3H, m), 3.9-4.1 (1H, m), 5.02 (2H, s), 5.56 (1.4H, br), 6.26 (2H, s), 6.44 (0.3H, t, J=9 Hz), 6.95 (0.6H, br), 7.12 (0.7H, t, J=9 Hz), 7.25-7.45 (5H, m), 7.36 (0.3H, d, J=6.6 Hz), 7.62 (0.7H, d, J=6.6 Hz), 8.50 (0.7H, s), 8.67 (0.3H, s).

MS (ES+): 349 (M+H), calcd for $C_{17}H_{24}N_4O_4$ 348.

Example 2

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide



By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-2-one.

Yield: 70 %

m.p.: 80-85 °C.

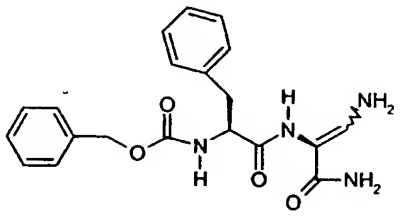
^{1}H-NMR: (DMSO-d_6), (ppm): 0.7-1.95 (13H, m), 3.9-4.15 (1H, m), 5.01 (2H, s), 5.45-5.65 (1.6H, br), 6.26 (2H, s), 6.43 (0.2H, t, J=10 Hz), 6.9-7.0 (0.4H, br), 7.12 (0.8H, t, J=10 Hz), 7.3-7.45 (5H, m), 7.58 (0.2H, d,

J=6.4 Hz), 7.62 (0.8H, d, J=6.4 Hz), 8.51 (0.8H, s), 8.66 (0.2H, s).

MS (ES+): 389 (M+H), calcd for $C_{20}H_{28}N_4O_4$ 388.

Example 3

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-acrylamide



By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-4-acetoxy-azetidin-2-one.

Yield: 71 %

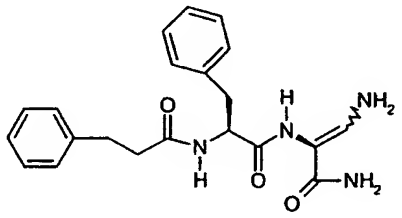
m.p.: 127-135 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 2.8-3.2 (2H, m), 4.2-4.4 (1H, m), 4.97 (2H, s), 5.3-5.55 (2H, br), 6.10 (2H, s), 7.14 (1H, t, J=11 Hz), 7.2-7.4 (10H, m), 7.78 (1H, d, J=6.4 Hz), 8.6 (1H, s).

MS (ES+): 383 (M+H), calcd for $C_{20}H_{22}N_4O_4$ 382.

Example 4

2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-amino-acrylamide



By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-4-acetoxy-azetidin-2-one.

Yield: 75 %

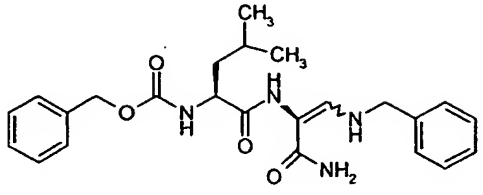
m.p.: 75-80 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 2.35-2.45 (2H, m), 2.70-3.15 (4H, m), 4.25-4.50 (1H, m), 5.25-5.50 (2H, br), 6.08 (2H, s), 7.06 (1H, t, J=11 Hz), 7.2-7.4 (10H, m), 8.32 (1H, d, J=6 Hz), 8.45 (1H, s).

MS (ES+): 381 (M+H), calcd for $C_{21}H_{24}N_4O_3$ 380.

Example 5

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in acetonitrile (5 ml) and water (1 ml), benzylamine (542 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 200 mg of the title compound was obtained as white solid.

Yield: 90%

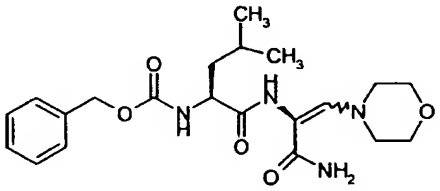
m.p.: 115-120 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.8 (9H, m), 3.9-4.1 (1H, m), 4.29 (2H, d, J=5.5 Hz), 4.9-5.1 (2H, m), 5.9-6.1 (0.7H, m), 6.28 (2H, s), 6.61 (0.3H, d, J=12 Hz), 7.17 (0.7H, d, J=12 Hz), 7.25-7.45 (10H, m), 7.60 (0.3H, d, J=6.5 Hz), 7.66 (0.7H, d, J=6.5 Hz), 8.35-8.55 (0.3H, m), 8.60 (0.7H, s), 8.72 (0.3H, s).

MS (ES+): 439 (M+H), calcd for $C_{24}H_{30}N_4O_4$ 438.

Example 6

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(morpholin-4-yl)-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in

acetonitrile (5 ml) and water (1 ml), morpholine (444 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the solid was washed with ether. 200 mg of the title compound was obtained as white solid.

Yield: 90%

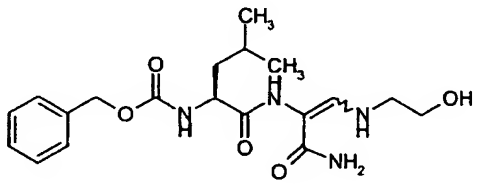
m.p.: 120-130 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-0.95 (6H, m), 1.3-1.75 (3H, m), 2.6-2.75 (4H, m), 3.5-3.6 (4H, m), 3.9-4.1 (1H, m), 5.00 (2H, s), 6.32 (2H, s), 7.07 (1H, s), 7.36 (5H, m), 7.67 (1H, d, J=6.7 Hz), 8.8 (1H, s).

MS (ES+): 419 (M+H), calcd for $C_{21}H_{30}N_4O_5$ 418.

Example 7

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(2-hydroxyethylamino)-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml), hydroethylamine (32 mg, 0.53 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel

column chromatography using methanol-chloroform as eluant. 70 mg of the title compound was obtained as white solid.

Yield: 70%

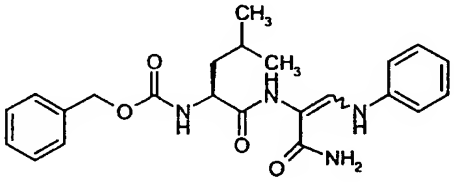
m.p.: 89-92 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-0.95 (6H, m), 1.4-1.75 (3H, m), 3.05-3.2 (2H, m), 3.35-3.5 (2H, m), 3.95-4.1 (1H, m), 4.65 (1H, t, J=5 Hz), 5.02 (2H, s), 5.4-5.55 (1H, m), 6.75 (2H, s), 7.12 (1H, d, J=12 Hz), 7.35 (5H, m), 7.65 (1H, d, J=6 Hz), 8.55 (1H, s).

MS (ES+): 393 (M+H), calcd for $C_{19}H_{28}N_4O_5$ 392.

Example 8

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-phenylamino-acrylamide



Aniline hydrochloride (500 mg, 3.8 mmole) was neutralised with Na_2CO_3 solution (600 mg, 5.7 mmole) and then extracted with ethyl acetate. After removal of solvent, aniline was dissolved in acetonitrile and added to a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml). The resulting mixture was stirred at room temperature overnight. After removal of solvent under vacuum and

lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.

Yield: 10%

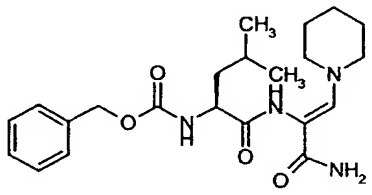
m.p.: 199-200 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.85-1.05 (6H, m), 1.5-1.8 (3H, m), 4.0-4.15 (1H, m), 5.09 (2H, s), 6.73 (2H, s), 6.88 (1H, t, J=7.2 Hz), 7.05 (2H, d, J=8 Hz), 7.24 (2H, d, J=7.5 Hz), 7.35 (5H, m), 7.7-7.9 (3H, m), 8.89 (1H, s).

MS (ES+): 425 (M+H), calcd for $C_{23}H_{28}N_4O_4$ 424.

Example 9

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-piperidino-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), piperidine (88 mg, 1.06 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 70 mg of the title compound was obtained as white solid.

Yield: 70%

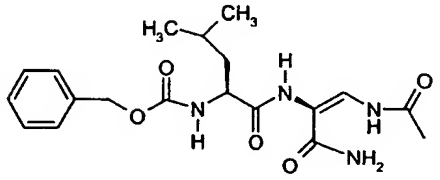
m.p.: 99-103 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.7 (15H, m), 3.2-3.3 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.23 (2H, s), 7.08 (1H, s), 7.3-7.4 (5H, m), 7.63 (1H, d, J=6 Hz), 8.76 (1H, s).

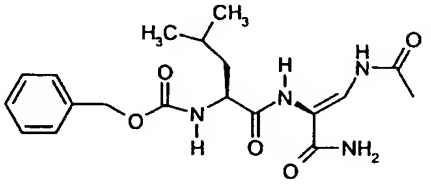
MS (ES+): 417 (M+H), calcd for $C_{22}H_{32}N_4O_4$ 416.

Example 10a and 10b

(2E)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide (10a)



(2Z)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide (10b)



150 mg (0.43 mmole) of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (from example 1) was dissolved in acetic anhydride (5 ml) and stirred at room temperature for 2 days. After removal of acetic anhydride, the residue was

purified by silica gel column chromatography using methanol-chloroform as eluant. 40 mg of the title compound (10a) and 45 mg of the title compound (10b) were obtained as white solid.

For (10a):

Yield: 24 %

m.p.: 73-76 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.45-1.75 (3H, m), 2.08 (3H, s), 3.95-4.1 (1H, m), 5.02 (2H, s), 6.90 (1H, br), 7.08 (1H, d, J=12 Hz), 7.3-7.4 (5H, m), 7.5 (1H, br), 7.70 (1H, d, J=6 Hz), 9.23 (1H, s), 10.98 (1H, d, J=12 Hz).

MS (ES+): 391 (M+H), calcd for $C_{19}H_{26}N_4O_5$ 390.

For (10b):

Yield: 27 %

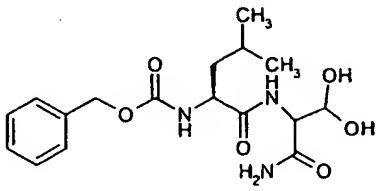
m.p.: 120-123 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.5-1.8 (3H, m), 2.01 (3H, s), 4.0-4.1 (1H, m), 5.06 (2H, s), 7.02 (2H, s), 7.3-7.45 (5H, m), 7.64 (1H, d, J=11.4 Hz), 7.84 (1H, d, J=6.2 Hz), 9.00 (1H, s), 9.13 (1H, d, J=11.4 Hz),

MS (ES+): 391 (M+H), calcd for $C_{19}H_{26}N_4O_5$ 390.

Example 11

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3,3-dihydroxy-propionamide



To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (30 mg, 0.086 mmole) (from example 1) in acetonitrile (2 ml) and water (0.5 ml), 5 drops of formic acid was added at 0 oC. The mixture was stirred at 0 oC for 1 hr. After removal of acetonitrile under vacuum, precipitate was formed by addition of water (2 ml). The solid was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.

Yield: 32%

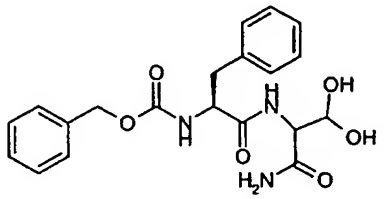
m.p.: 86-90 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.4-1.75 (3H, m), 4.0-4.3 (2H, m), 4.6-4.8 (1H, m), 5.02 (1H, s), 5.03 (1H, s), 6.35-6.45 (1H, m), 6.6-6.7 (1H, m), 7.2 (2H, br), 7.3-7.4 (5H, m), 7.5-7.9 (2H, m).

MS (ES+): 350 (M-H_2O+H), calcd for $C_{17}H_{25}N_3O_6$ 367.

Example 12

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3,3-dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide (from example 2).

Yield: 45%

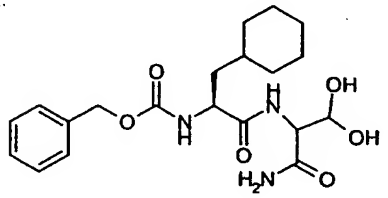
m.p.: 105-110 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.8 (13H, m), 4.0-4.3 (2H, m), 4.6-4.85 (1H, m), 5.03 (2H, m), 6.3-6.7 (2H, m), 7.14 (2H, br), 7.3-7.4 (5H, m), 7.45-7.9 (2H, m).

MS (ES+): 390 (M-H_2O+H), calcd for $C_{20}H_{29}N_3O_6$ 407.

Example 13

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3,3-dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-acrylamide (from example 3).

Yield: 40%

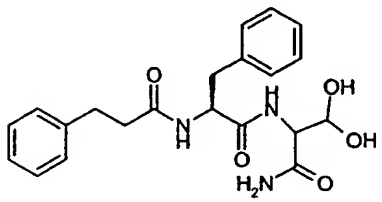
m.p.: 98-103 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 2.7-3.15 (2H, m), 4.2-4.5 (2H, m), 4.65-4.8 (1H, m), 4.94 (2H, s), 6.35-6.5 (1H, m), 6.6-6.75 (1H, m), 7.1-7.45 (12H, m), 7.5-7.65 (1H, m), 7.9-8.15 (1H, m).

MS (ES+): 384 (M-H_2O+H), calcd for $C_{20}H_{23}N_3O_6$ 401.

Example 14

2-[2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido]-3,3-dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido)-3-amino-acrylamide (from example 4).

Yield: 48%

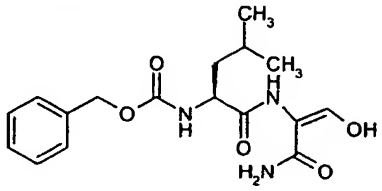
m.p.: 105-110 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 2.3-2.4 (2H, m), 2.6-3.2 (4H, m), 4.2-4.3 (1H, m), 4.5-4.8 (2H, m), 6.3-6.45 (1H, m), 6.55-6.7 (1H, m), 7.1-7.45 (12H, m), 7.85-8.35 (2H, m).

MS (ES+): 382 (M-H_2O+H), calcd for $C_{21}H_{25}N_3O_5$ 399.

Example 15

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-hydroxy-acrylamide



To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (55 mg, 0.158 mmole) (from example 1) in acetonitrile (3 ml) and water (0.5 ml), 10 drops of formic acid was added at 0 oC. The mixture was stirred at room temperature for 1 hr. After removal of solvent under vacuum, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 20 mg of the title compound was obtained as white solid.

Yield: 36%

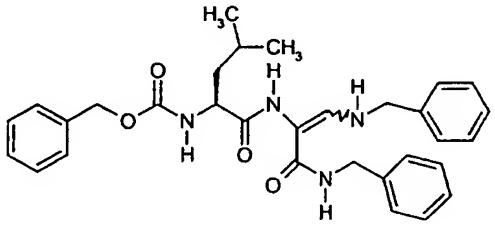
m.p.: 105-115 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.4-1.8 (3H, m), 3.9-4.15 (1H, m), 5.02 (2H, s), 6.5-7.1 (1.5H, m), 7.3-7.5 (7H, m), 7.65-7.8 (1H, m), 9.05 (1H, s), 10.2 (0.5H, m).

MS (ES+): 350 (M+H), calcd for $C_{17}H_{23}N_3O_5$ 349.

Example 16

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-N-benzyl-acrylamide



A solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-phenoxy-azetidin-2-one (1.1 g, 3.3 mmole) in ethanol (20 ml) and 5 ml of benzylamine was stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 0.96 g of the title compound was obtained as white solid.

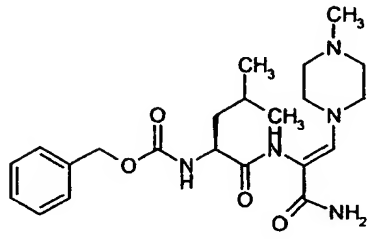
Yield: 55%

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.4-1.8 (3H, m), 3.9-4.1 (1H, m), 4.2-4.4 (4H, m), 4.7-4.9 (2H, m), 6.0-6.2 (0.5H, m), 6.6-6.8 (1H, m), 7.1-7.4 (16H, m), 7.6-7.8 (1H, m), 8.3-8.5 (0.5H, m), 8.68 (0.5H, s), 8.85 (0.5H, s).

MS (ES+): 529 (M+H), calcd for $C_{31}H_{36}N_4O_4$ 528.

Example 17

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(4-methylpiperazino)-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), 4-methylpiperazine (106 mg, 1.06 mmole) was added and stirred at room temperature overnight. After removal of acetonitrile under vacuum, the residue was dissolved in ethyl acetate and washed with water, brine and dried with Na_2SO_4. After removal of solvent, 30 mg of the title compound was obtained as white solid.

Yield: 27%

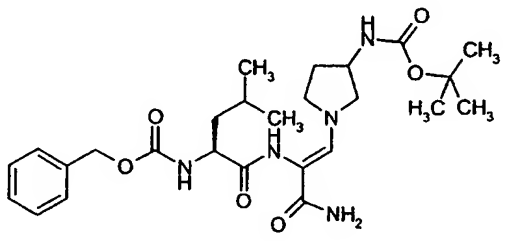
m.p.: 93.5-95 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.3-1.8 (3H, m), 2.13 (3H, s), 2.2-2.35 (4H, m), 3.2-3.35 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.29 (2H, s), 7.07 (1H, s), 7.3-7.4 (5H, m), 7.66 (1H, d, J=6.7 Hz), 8.79 (1H, s).

MS (ES+): 432 (M+H), calcd for $C_{22}H_{33}N_5O_4$ 431.

Example 18

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(3-tert-butoxycarbonylamino-pyrrolidino)-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), 3-tert-butoxycarbonylamino-pyrrolidine (239 mg, 1.28 mmole) was added and stirred at room temperature overnight. After removal of acetonitrile under vacuum, the residue was dissolved in ethyl acetate and washed with water, brine and dried with Na_2SO_4. After removal of solvent, 120 mg of the title compound was obtained as white solid.

Yield: 90%

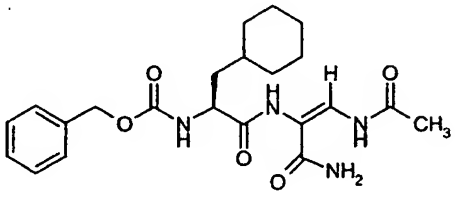
m.p.: 140-142 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.0 (6H, m), 1.3-2.0 (5H, m), 1.37 (9H, s), 3.0-3.2 (1H, m), 3.3-3.7 (3H, m), 3.9-4.1 (2H, m), 5.01 (2H, s), 6.25 (2H, s), 7.12 (1H, d, J=6.5 Hz), 7.22 (1H, s), 7.3-7.4 (5H, m), 7.66 (1H, d, J=6.5 Hz), 8.75 (1H, s).

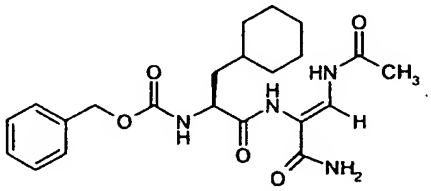
MS (ES+): 518 (M+H), calcd for $C_{26}H_{39}N_5O_6$ 517.

Example 19a and 19b

(2E)-(2S-2-benzyloxycarbonylamino-2-cyclohexymethyl-acetamido)-3-acetamido-acrylamide (19a)



(2Z)-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-acetamido-acrylamide (19b)



By a similar method as described in example 10, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide (example 2).

For (19a):

Yield: 21 %

m.p.: 140-142 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.7-1.8 (13H, m), 2.01 (3H, s), 3.95-4.1 (1H, m), 5.02 (2H, m), 6.88 (1H, br), 7.07 (1H, d, J=11 Hz), 7.3-7.4 (5H, m), 7.5 (1H, br), 7.65 (1H, d, J=6 Hz), 9.20 (1H, s), 10.97 (1H, d, J=11 Hz).

MS (ES+): (M+H), calcd for $C_{22}H_{30}N_4O_5$ 430.

For (19b):

Yield: 41 %

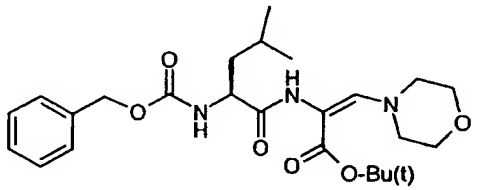
m.p.: 151-153 °C.

^{1}H-NMR: (DMSO-d_6), (ppm): 0.8-1.8 (13H, m), 1.99 (3H, s), 4.0-4.1 (1H, m), 5.04 (2H, m), 7.01 (2H, br), 7.3-7.4 (5H, m), 7.62 (1H, d, J=11.4 Hz), 7.81 (1H, d, J=6.2 Hz), 8.98 (1H, s), 9.11 (1H, d, J=11.4 Hz),

MS (ES+): (M+H), calcd for $C_{22}H_{30}N_4O_5$ 430.

Example 20

tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (20)



A mixture of ZLeuGlyOBu^t (0.5g, 0. mmol), 1-(diethoxymethyl)imidazole (0.36g, mmol) and camphor shulphonic acid (0.056g, mmol) in toluene was treated with morpholine (1.0ml, mmol) and was refluxed for 24hrs. Solvent was removed *in vacuo* and the crude product obtained was purified over silica gel column chromatography using a gradient mixture of hexane and ethyl acetate (1:1 to 2:1) gave 20 mg of title compound.

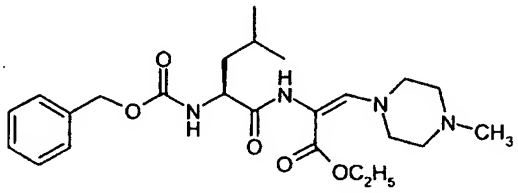
Yield; 3.2%

^{1}H NMR (DMSO- d_6): δ 0.80-0.90(m, 6H), 1.24-1.72(m, 3H), 3.2-3.48(m, 8H), 3.95-4.06(m, 1H), 5.00(AB_q, 2H, J= 2.7

and 13.0Hz), 7.11(s, 1H), 7.34(s, 5H), 7.43(d, 1H, J= 8.0Hz), 8.49(s, 1H).

Example 21

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate (21)



A mixture of ZLeuGlyOEt (0.3g, 0.86mmol), 1-(diethoxymethyl)imidazole (0.28g, 1.27mmol) in toluene was treated with N-methylpiperazine(0.94ml, 8.5mmol) and was refluxed for 24hrs. Solvent was removed *in vacuo* and the crude product obtained was purified over. Purification of the above crude product over silica gel column chromatography using a mixture of ethyl acetate and methanol (9:1) gave the title compound (0.025g),

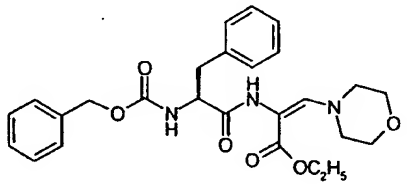
Yield 6.3%,

m. p. 184°C.

^{1}H NMR (DMSO- d_6): δ 0.87-0.95(m, 6H), 1.12(t, 3H, J= 7.0Hz), 1.43-1.79(m, 3H), 2.11(s, 3H), 2.21(s, 4H), 3.40(m, 4H), 3.94(q, 2H, J= 7.0Hz), 3.97-4.12(m, 1H), 5.01(AB_q, 2H, J= 8.3 and 12.6Hz), 7.20(s, 1H), 7.34(s, 5H), 7.44(d, 1H, J= 7.9Hz), 8.53(s, 1H).

Example 22

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (22)



By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

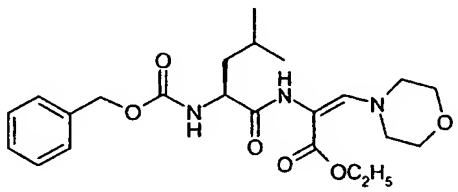
Yield; 4.5%,

m.p. 215-217 ^{0}C

^{1}H NMR (DMSO-d6): δ 1.14(t, 3H, J= 7.1Hz), 2.69-3.10(m, 2H), 3.35-3.47(m, 8H), 3.98(q, 2H, J= 6.2Hz), 4.20-4.33(m, 1H), 4.93(AB_q, 2H, J= 6.7 and 12.7Hz), 7.20-7.40(m, 11H), 7.57(d, 1H, J= 8.4Hz), 8.79(s, 1H).

Example 23

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (23)



By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-

(benzyloxycarbonylamino)-2- isopropylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole

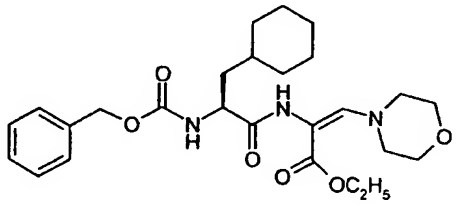
Yield; 16%,

m.p. 139-141 ^{0}C

^{1}H NMR (DMSO-d6): δ 0.87(t, 6H, J= 6.1Hz), 1.13(t, 3H, J= 7.1Hz), 1.43-1.78(m, 3H), 3.38-3.51(m, 8H), 3.90-4.09(m, 3H), 5.00(AB_q, 2H, J= 2.1 and 12.7Hz), 7.21(s, 1H), 7.34(s, 5H), 7.45(d, 1H, J= 7.5Hz), 8.56(s, 1H).

Example 24

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (24)



By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-(benzyloxycarbonylamino)-2- cyclohexylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

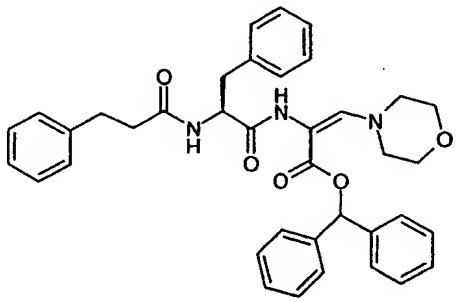
Yield; 10%,

m.p. 202^{0}C

^{1}H NMR (DMSO-d6): δ 0.75-1.76(m, 16H), 3.35-3.50(m, 8H), 3.90-4.12(m, 3H), 5.00(s, 2H), 7.21(s, 1H), 7.34(s, 5H), 7.45(d, 1H, J= 7.0Hz), 8.54(s, 1H).

Example 25

Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate



By a similar method as described in example 21, the title compound compound was obtained from Diphenylmethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

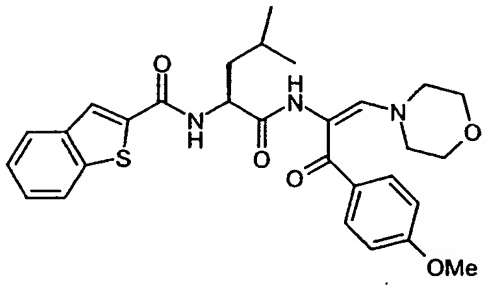
Yield; 27%,

m.p. 167-170 ^{0}C

^{1}H NMR (DMSO-d6): 2.32 (t, 2H, J= 8.4Hz), 2.67(t, 2H, J= 7.0Hz), 2.70-3.26(m, 2H), -3.40-3.55(m, 8H), 4.50-4.67(m, 1H), 6.74(s, 1H), 7.10-7.49(m, 20H), 8.27(d, 1H, J= 8.0Hz), 8.95(brs, 1H).

Example 26

2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone (26).



A mixture of N-(benzothiophene-2carbonyl)amino-Leucine (0.332g, 1.14mmol), DCC(0.235g, 1.14mmol) and 1-hydroxy benzotriazole (0.154g, 1.14mmol) in dry THF was stirred under nitrogen at r.t. for 1h and cooled to 0 °C. The suspension obtained was filtered and to the filtrate was added 2-amino-4'-methoxy acetophenone (0.23g, 1.14mmol) followed by triethyl amine (0.127g, 1.25mmol). The reaction mixture was stirred at r.t. for 6 hrs. and evaporated *in vacuo* to give the crude product. Purification of the above crude product over silica gel column chromatography using a mixture of hexane and ethyl acetate (2:3) gave 288 mg of title compound 2[2S-(benzothiophene-2-carbonyl)amino-2-isopropylmethyl-acetamido]-(4'methoxy)acetophenone.

Yield; 58%,

^{1}H NMR (DMSO- d_6): δ 0.90-1.00(m, 6H), 1.58-1.82(m, 3H), 3.84(s, 3H), 4.55-4.67(m, 3H), 7.05(d, 2H, J=8.8Hz), 7.44-

7.48(m, 2H), 7.95-8.05(m, 4H), 8.28(s, 1H), 8.35(t, 1H, J=5.9Hz), 8.86(d, 1H, J=8.3Hz).

A mixture of 2-[2S-(benzothiophene-2-carbonyl)-amino-2-isopropylmethyl-acetamido]-4'methoxyacetophenone (0.271g, 0.618mmol) and 1-(diethoxymethyl)imidazole (0.202g, 0.927mmol) in toluene was treated morpholine (0.269g, 3.09mmol) and refluxed at 130 ^{0}C over 22 hrs. Toluene was removed in vacuo and the crude product was purified over silica gel column chromatography using a mixture of ethyl acetate and methanol (9:1) to give 200 mg of title compound.

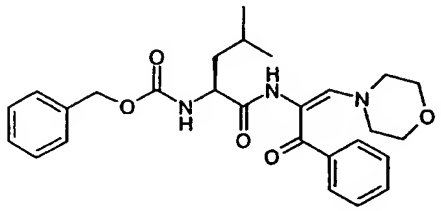
Yield : 60%.

m.p. 134-136 ^{0}C

^{1}H NMR (DMSO- d_6): δ0.82-0.93(m, 6H), 1.15-1.87(m, 3H), 3.40-3.56(m, 8H), 3.75(s, 3H), 4.37-4.50(m, 1H), 6.90(d, 2H, J=8.6Hz), 7.09(s, 1H), 7.41-7.47(m, 4H), 7.95-8.05(m, 2H), 8.25(s, 1H), 8.81(d, 1H, J=7.7Hz), 8.98(s, 1H).

Example 27

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 2-(morpholino-1-methylenyl)-acetophenone (27).



By following the procedure as described in example 25, the title compound was obtained from2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-acetophenone, morpholine and 1-(diethoxymethyl)imidazole.

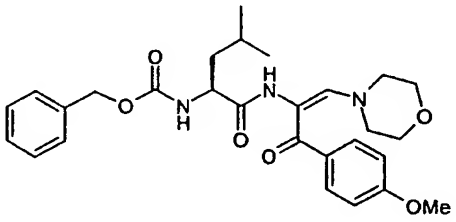
Yield; 53%,

m.p. 107-109 ^{0}C

^{1}H NMR (DMSO-d_6): δ 0.78-0.85(m, 6H), 1.06-1.63(m, 3H), 3.40-3.55(m, 8H), 3.90-4.05(m, 1H), 5.00(AB_q, 2H, J= 2.0 and 10.7Hz), 7.13(s, 1H), 7.35(s, 5H), 7.38s, 5H), 7.44(d, 1H, J= 8.0Hz), 8.81(s, 1H).

Example 28

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 2-(morpholino-1-methylenyl)-4'-methoxyacetophenone (28).



By following the procedure as described in example 25, the title compound was obtained from2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 4'-methoxyacetophenone, morpholine and 1-(diethoxymethyl)imidazole.

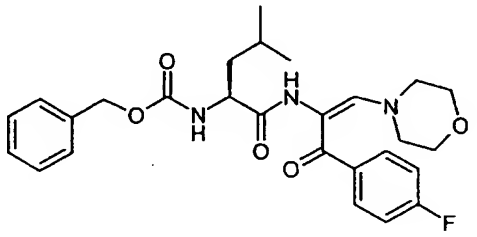
Yield; 53%,

m.p. 140-143 ^{0}C

^{1}H NMR (DMSO-d_6): δ 0.77-0.85(m, 6H), 1.17-1.63(m, 3H), 3.40-3.53(m, 8H), 3.75(s, 3H), 3.90-4.05(m, 1H), 4.99(s 2H), 6.89(d, 2H, J= 8.6Hz), 7.07(s, 1H), 7.33-7.45(s, 8H), 8.80(s, 1H).

Example 29

2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-fluoroacetophenone (29).



By following the procedure as described in example 25, the title compound was obtained from2-[2S-(Benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]- 4'-fluoroacetophenone, morpholine and 1-(diethoxymethyl)imidazole.

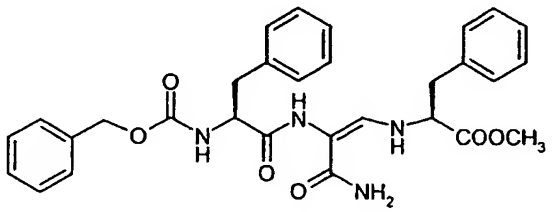
Yield; 30%,

m.p. 156-157 ^{0}C

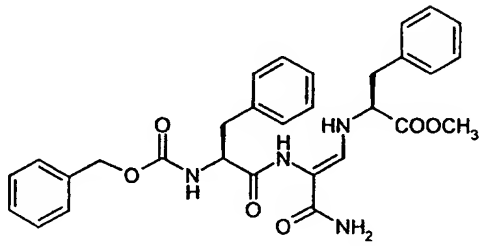
^{1}H NMR (DMSO-d_6): δ 0.80-0.90(m, 6H), 1.05-1.64(m, 3H), 3.50(m, 8H), 3.87-4.03(m, 1H), 5.00(s,2H), 7.15-7.50(m, 11H), 8.85(s, 1H).

Example 30a and 30b

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide (30a)



(2Z)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide (30b)



By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of phenylalanine.

For 30a:

Yield; 34%,

m.p. 71-73 ^{0}C

NMR(DMSO-d_6): 2.75-3.03(m, 4H), 3.63(s, 3H), 4.12-4.38(m, 2H), 4.96(s, 2H), 6.00 and 6.55(2br. S, 2H), 6.34(d, 1H,

J= 12.2Hz), 7.20-7.46(m, 15H), 7.67(d, 1H, J= 6.0Hz), 8.30-8.40(m, 1H), 8.70(s, 1H).

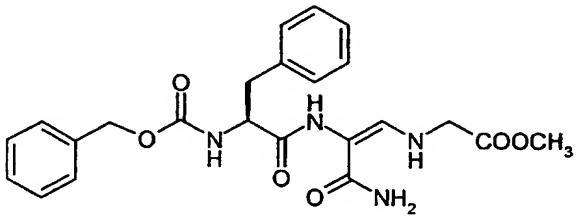
For 30b:

Yield; 42%, m.pt. 85-87 °C

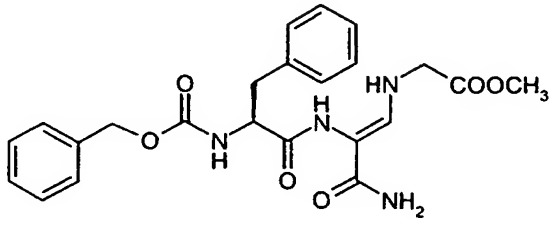
NMR(DMSO-d_6): 2.79-3.15(m, 4H), 3.60(s, 3H), 4.16-4.35(m, 2H), 4.96(AB_q, 2H, J= 2.2 and 12.6Hz), 5.42-5.53(m, 1H), 6.15(brs, 2H), 7.08(d, 1H, J= 13.0Hz), 7.20-7.30(m, 15H), 7.79(d, 1H, J= 6.5Hz), 8.71(s, 1H).

Example 31a and 31b

(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide (31a)



(2E)-(2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide (31b)



By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-

2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of glycine

For 31a:

Yield; 36%,

m.p. 108-110 °C

NMR(DMSO-d_6):1.21(t, 3H, J= 7.0Hz), 2.76-3.04(m, 2H), 3.94(d, 2H, J= 6.2Hz), 4.11(q, 2H, J= 7.0Hz), 4.11-4.25(m, 1H), 4.96(s, 2H), 6.02 and 6.53(2brs, 2H), 6.30(d, 1H, J= 12.5Hz), 7.26-7.35(m, 10H), 7.67(d, 1H, J= 6.9Hz), 8.12-8.25(m, 1H), 8.72(s, 1H).

For 31b:

Yield; 41%,

m.p. 145-147 °C

NMR(DMSO-d_6): 1.20(t, 3H, J= 7.4Hz), 2.75-3.15(m, 2H), 3.88(d, 2H, J= 5.9Hz), 4.16(q, 2H, J= 7.4Hz), 4.25-4.87(m, 1H), 4.98(s, 2H), 5.22-5.35(m, 1H), 6.20(brs, 1H), 7.05(d, 1H, J= 11.8Hz), 7.19-7.35(m, 10H), 7.75(d, 1H, J= 6.6Hz), 8.70(s, 1H).

Biological Example

Testing of inhibitors for inhibition of cathepsin B, L, K and S.

In vitro assay procedure for cathepsin B

The compounds of formula I were tested for inhibition of cathepsin B using the known method (A.J. Barret et al., Biochem. J. 1982, 201, 189-198). To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin B, diluted to give approximate 10 F units/min, buffer: 56 mM sodium

acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) a 10 μL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μl of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin L

To a 170 μl of enzyme-buffer mixture (enzyme: r rat cathepsin L, diluted to give approximate 15 F units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) a 10 μL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 μl of 1 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin K

To a 170 μl of enzyme-buffer mixture (enzyme: r cathepsin K, diluted to give approximate 30 F units/min,

buffer: 100 mM sodium acetate, 5 mM EDTA, 20 mM L-cysteine, 0.01% Brij, pH 5.5) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µl of 2.7 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin S

To a 170 µl of enzyme-buffer mixture (enzyme: r cathepsin S, diluted to give approximate 30 F units/min, buffer: 100 mM sodium phosphate, 1 mM EDTA, 5 mM DTT, 0.01% Brij, pH 6.5) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µl of 1.2 mM substrate (N-CBZ-Val-Val-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

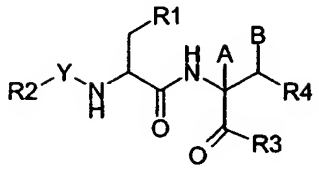
Table 1. In vitro inhibitory activity of compounds on cysteine proteases

Example No.	IC_{50} (μM)			
	Cathepsin B	Cathepsin L	Cathepsin K	Cathepsin S
1	4.35	0.094	0.011	0.069
2	1.17	0.072	1.78	0.026
3	1.42	0.0055	0.23	0.26
4	2.63	0.015	1.2	0.0087
5	2.28	0.064	0.0031	0.061
6	0.37	0.075	0.003	0.05
7	1.96	0.23	0.012	0.18
8	37.1	2.36	0.33	6.9
9	0.89	0.062	0.014	0.05
10a	45.21	8.62	1.42	0.013
10b	50.51	>64	10.73	>3.2
11	2.4	0.11	0.014	0.015
12	1.64	0.076	2.06	0.0035
13	1.4	0.004	0.4	0.004
14	0.98	0.004	1.13	0.004
15	9.5	0.17	0.018	0.075
16	0.15	0.015	0.01	0.026
17	1.9	0.23	0.019	0.18
18	1.9	0.12	0.0096	0.039

19a	>58	>58	>58	1.86
19b	>58	58	>58	5.23
20	0.42	0.08	0.004	0.38
21	8.21	0.21	0.087	0.3
22	0.08	0.04	0.04	0.42
23	0.45	0.089	0.011	0.038
24	0.082	0.082	0.37	0.0033
25	0.06	0.18	0.065	0.18
26	1.47	0.075	0.015	0.99
27	32.27	4.24	0.14	52.2
28	1.96	0.27	0.016	1.3
29	50.3	2.01	0.34	50.3
30a	1.83	0.048	0.28	0.57
30b	1.83	0.035	0.28	1.21
31a	0.66	0.017	0.13	0.7
31b	0.43	0.017	0.09	0.43

CLAIMS

1. A compound of formula (I)



(I)

wherein:

Y represents -C(O)- or -S(O_2)-;

R_1 represents a radical of formula R_6-(ALK)$_p$-(Z)$_n$-(ALK)$_q$- wherein Z represents -O- or -S-, ALK represents a divalent C_1-C_3alkyl or halogen-substituted C_1-C_3alkyl radical, p and q are independently 0 or 1, n is 0 or 1 when q is 1 and n is 0 when q is 0, and R_6 is hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group; or R_1 together with the carbon atom to which it is attached forms a cycloalkyl ring;

R_2 represents -OR_5 or -R_5;

R_5 represents a radical of formula R_7-(A)$_t$- wherein t is 0 or 1; A represents (i) an optionally substituted divalent C_1-C_6alkyl, radical which may be interrupted by one or more non-adjacent -O-, -S- or -NH- linkages, or (ii) a divalent C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl,

cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R_7 represents hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

R_3 represents (I) an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR_8 or $N(R_8)_2$ or (iii) OR_8 wherein R_8 represents hydrogen or an optionally substituted C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, cycloalkyl, cycloalkenyl or aryl;

A and B taken together represent a bond and R_4 represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R_4 each independently represents a hydroxy or substituted hydroxy group;

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

2. A compound as claimed in claim 1 wherein Y is -C(O)-.

3. A compound as claimed in claim 1 or claim 2 wherein R_1 is a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy, methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy -phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl;

tetrahydroquinolinyl; tetrahydronaphthyl; aminonaphthyl; or acetamidonaphthyl.

4. A compound as claimed in claim 1 or claim 2 wherein R_1 phenyl, isopropyl, cyclohexyl or 3-pyridinyl.

5. A compound as claimed in any of the preceding claims wherein R_2 is benzyloxy, 3-phenylpropyloxy, 3-phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl, methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-prop-1-enyl.

6. A compound as claimed in any of claims 1 to 4 wherein R_3 may be, for example, methyl, ethyl, isopropyl, t-butyl, cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl, pyridyl, $-NH_2$, methylamino, dimethylamino, benzylamino, piperidino, morpholino, piperazino, N-methylpiperazino, methoxy, ethoxy, t-butyloxy or phenoxy.

7. A compound as claimed in any of the preceding claims wherein A and B taken together represent a bond, and R_4 is $-NH_2$, acetylamino, methylamino, dimethylamino, benzylamino, morpholinyl, piperidino, morpholino, piperazino or N-methylpiperazino, (methoxycarbonyl)-methylamino, (methoxycarbonyl)-phenethylamino, -OH, methoxy, allyloxy, benzyloxy.

8. A compound as claimed in any of claims 1 to 6 wherein A represents hydrogen and B and R_4 each independently represents a hydroxygroup.

9. A compound as claimed in claim 1 which is specifically named and characterised in any of the Examples herein.

10. A pharmaceutical composition containing a compound as claimed in any of the preceding claims and a pharmaceutically acceptable carrier.

11. The use of a compound as claimed in any of claims 1 to 9 in the preparation of a composition for inhibiting cysteine protease activity in the body of a mammal suffering a disease mediated by such activity.

12. A method of treatment of an animal suffering from a disease mediated by cysteine protease activity, which method comprises administering to the mammal a sufficient amount of a compound as claimed in any of claims 1 to 9 to inhibit such activity.

13. The use as claimed in claim 11 or a method as claimed in claim 12 wherein the disease is muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, or protozoal or bacterial disease.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agents: **WALSH, David, Patrick** et al.; Appleyard Lees, 15 Clare Road, Halifax HX1 2HY (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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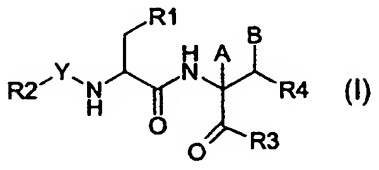
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/09169 A3

(54) Title: CYSTEINE PROTEASE INHIBITORS



(I)

(57) Abstract: This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins. A compound of formula (I) is described or a pharmaceutically acceptable salt, hydrate or solvate thereof. Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

INTERNATIONAL SEARCH REPORT

In ıtional Application No
PCT/GB 00/02830

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K5/062 C07K5/065 C07D295/12 A61K38/05 A61K31/5375
A61K31/381 A61P19/00 A61P31/00 A61P35/00 A61P37/00
A61P9/00 A61P25/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DORAN, JOHN D. ET AL.: "DEACYLATION AND REACYLATION FOR A SERIES OF ACYL CYSTEINE PROTEASES INCLUDING ACYL GROUPS DERIVED FROM NOVEL CHROMOPHORIC SUBSTRATES" BIOCHEMISTRY (1996) 35(38) 12487-12494, XP002163740 ---	
A	WO 96 32408 A (SYNPHAR LAB INC) 17 October 1996 (1996-10-17) cited in the application ---	
A	WO 98 12176 A (SYNPHAR LAB INC) 26 March 1998 (1998-03-26) cited in the application ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2001	05/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cervigni, S

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/GB 00/02830

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BAGGIO RICKY ET AL: "From poor substrates to good inhibitors: Design of inhibitors for serine and thiol proteases." BIOCHEMISTRY, vol. 35, no. 11, 1996, pages 3351-3353, XP002163741 ISSN: 0006-2960	
A	DUFFY K J ET AL: "Design and synthesis of diaminopyrrolidinone inhibitors of human osteoclast cathepsin K" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,GB,OXFORD, vol. 9, no. 14, 19 July 1999 (1999-07-19), pages 1907-1910, XP004171607 ISSN: 0960-894X	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ational Application No
PCT/GB 00/02830

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 4951896 A	30-10-1996
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		DE 69610978 D	21-12-2000
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		US 5986108 A	16-11-1999
WO 9812176 A	26-03-1998	AU 4133697 A	14-04-1998
		EP 0950046 A	20-10-1999
		US 5959123 A	28-09-1999